Interaction of synthetic signal sequence fragments with model membranes

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Peptide fragments corresponding to the signal sequence of chicken lysozyme, labelled with the fluorescent 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) group have been synthesized. The emission characteristics and fluorescence polarization of the dansyl group have been used to study the interaction of signal sequence fragments with liposomes. The peptide fragments bind to liposomes and are associated with the hydrophobic core of the bilayer.

Signal sequence *Synthetic peptide* *Fluorescence* *Model membrane*

1. INTRODUCTION

Many secreted and transmembrane proteins are biosynthesized as precursors with amino terminal extensions of 15–30 residues [1,2]. These extensions or 'signal sequences' generally have a positively charged amino terminus followed by a central hydrophobic core segment. The manner in which signal sequences initiate transfer of proteins across membranes has been the subject of intense research recently [1–11]. The transfer process has been studied in intact cells and cell-free protein synthesis reactions. Due to the chemical complexity of cells and cell-free systems, assessment of the individual contributions of signal sequences, processing enzymes and membrane components to the export process becomes difficult. Studies using isolated preproteins and signal sequences are few [12–15], presumably due to the difficulty in isolating precursor proteins and signal sequences. Chemical synthesis would be a convenient method for generating signal sequences in sufficient amounts to enable detailed structural and physico-chemical studies. The use of a synthetic approach has been restricted to a circular dichroism study of the signal sequence of preproparathyroid hormone [13]. Here, a study is presented on the interaction of chemically synthesized signal sequence fragments of chicken lysozyme (I) [16], labelled with the fluorescent dansyl group, with model membranes.

Met–Arg-Ser-Leu-Leu-Ile-Leu-Val-Leu-Cys-Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly (I)

Abbreviations: Boc, *t*-butyloxycarbonyl; Bzl, benzyl; dans, 5-dimethylaminonaphthalene-1-sulfonyl; DMPC, dimyristoylphosphatidylcholine; EPC, egg phosphatidylcholine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; OBzl, benzyl ester; OMe, methyl ester

2. EXPERIMENTAL

Boc–Ser-Leu-Leu-Ile-OMe, Boc–Leu-Val-Leu-OMe, Boc–Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-OBzl, Boc–Cys(Bzl)-Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-OBzl, Boc–Leu-Val-Leu-Cys(Bzl)-Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-OBzl and Boc–Leu-Val-Leu-Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-OBzl were synthesized by solution phase methods using dicyclohexylcarbodiimide-1-hydroxybenzotriazole-mediated couplings. All compounds were chromatographically homogeneous on silica gel and yielded satisfactory amino acid analyses. The corresponding dansyl peptides were prepared by removal of the Boc group with 90% formic acid, followed by treatment with dansyl chloride and triethylamine

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in methanol. The dansylated peptide esters were purified by TLC on silica gel with the solvent system 10% methanol/chloroform. The dansyl peptide esters were converted to their corresponding amides by dissolving them in methanol saturated with ammonia. Detailed synthetic procedures will be reported elsewhere.

EPC and DMPC were from Sigma (St. Louis). EPC was purified as in [17]. DMPC was homogeneous on TLC and was used without further purification. Small unilamellar vesicles of EPC and DMPC were prepared as follows: a chloroform solution of the lipid was dried under nitrogen and the resultant film of the phospholipid was dispersed in 5 mM Hepes (pH 7.0) and sonified to clarity. For DMPC vesicles, sonication was done above the phase transition temperature.

Fluorescence spectra were recorded on a Hitachi 650-10S fluorescence spectrometer, operated in the normal mode with 3 nm excitation and emission bandpass. All spectra are uncorrected. Dansylated peptides in ethanol were added to aqueous solution and emission spectra were recorded at 30°C with $\lambda_{ex} = 330$ nm. Alcohol concentration was kept below 1%. The polarization accessory to the Hitachi 650-10S was used for polarization measurements.

3. RESULTS AND DISCUSSION

The emission characteristics of the dansyl group are sensitive to microenvironment [18] and have been used to study peptide–lipid interactions and aggregation [19–22]. Here, the emission characteristics and polarization of fluorescence of the dansyl group have been used to study the interaction of signal sequence fragments with lipid vesicles.

The emission spectra of dans–Leu-Val-Leu-NH_2, *1*, dans–Ser-Leu-Leu-Ile-NH_2, *2* and dans-Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-NH_2, *3* at 5 μM concentration in 5 mM Hepes (pH 7.0) and in the presence of EPC small unilamellar vesicles (200 μg/ml) are shown in fig.1. A blue shift in the emission maximum and an enhancement in intensity are observed in the presence of liposomes. This suggests that the peptides bind to the liposomes. A plot of the ratio of emission intensities (R) at 510 nm to that at 550 nm *vs* molar ratio of lipid to peptides is shown in fig.2. Clearly, the octapeptide *3* binds more strongly to the liposomes as compared to the tri- and tetrapeptides *1* and *2*. Similar shifts in the emission maxima and enhancement in intensity were observed with DMPC vesicles.

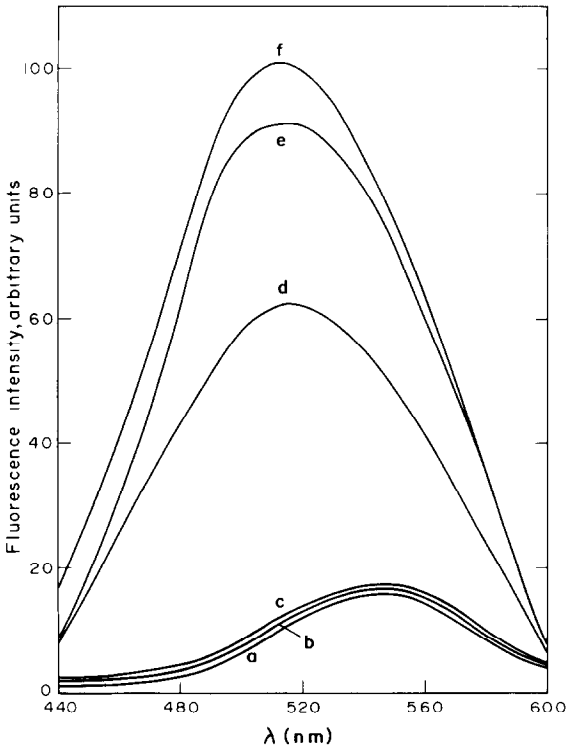

Fig.1. Uncorrected fluorescence emission spectra of peptides (5 μM) in 5 mM Hepes buffer (pH 7.0) and in the presence of EPC vesicles (200 μg/ml). (a) dans–Leu-Val-Leu-NH_2, *1*; (b) dans–Ser-Leu-Leu-Ile-NH_2, *2*; (c) dans–Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-NH_2, *3*; (d) *1* + lipid vesicles; (e) *2* + lipid vesicles; (f) *3* + lipid vesicles.

To determine whether the octapeptide *3* was associated with the hydrophobic core of the bilayer, the fluorescence polarization value P was measured in the gel and liquid crystalline phases of DMPC vesicles. At 30°C (the liquid crystalline phase) $P = 0.17$ whereas at 10°C (the gel phase) $P = 0.27$. The sensitivity of P to the physical state of the bilayer suggests that the bound peptide is associated with the hydrophobic core of the bilayer.

The longer peptides dans–Cys(Bzl)–Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-NH_2, *4* and dans–Leu-Val-Leu-Cys(Bzl)-Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-NH_2, *5* show an emission maximum at 480 nm in buffer indicating that they are strongly aggre-

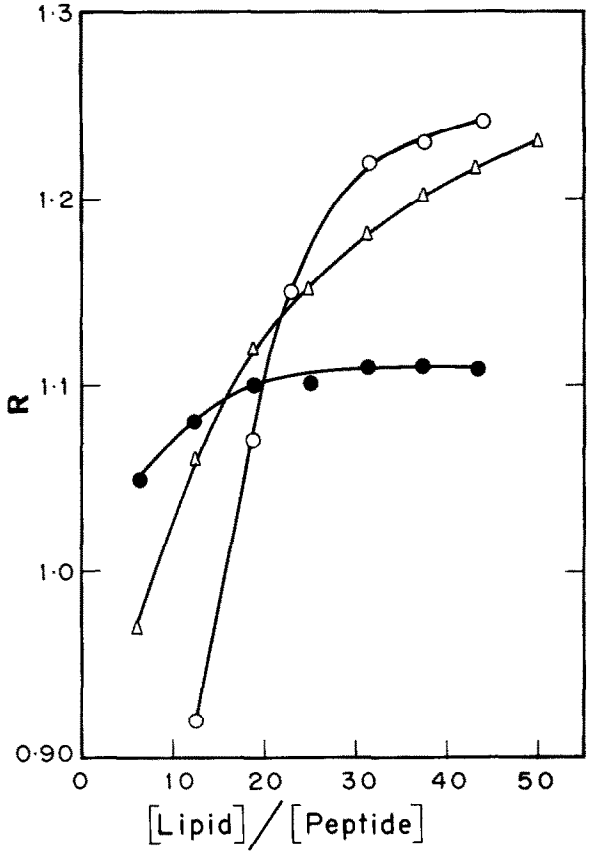

Fig.2. Ratio of fluorescence intensity at 510 nm and 550 nm (R) as a function of lipid : peptide molar ratio. (Δ—Δ) dans–Leu-Leu-Ile-NH_2. (○—○) dans–Ser-Leu-Leu-Ile-NH_2. (●—●) dans–Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-NH_2.

gated. It is unlikely that the S–Bzl group contributes significantly to the aggregation as dans–Leu-Val-Leu-Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly-NH_2, *6* also has an emission maximum at 480 nm. No changes were observed in the fluorescence spectrum of the peptides *5* and *6* in the presence of DMPC vesicles. However, change in polarization value was observed above and below the phase transition temperature. At 30°C $P = 0.18$ and at 10°C $P = 0.25$. P was constant in the range 30–10°C for the peptides in buffer. Thus the longer peptides have a tendency to aggregate and bind to the liposomes. Such binding is not reflected in the emission spectrum since the transfer of the dansyl group from an aggregated phase to a membrane phase may not result in a significant change of environment.

Genetic studies in *E.coli* have shown the importance of hydrophobic amino acid residues in the export of periplasmic and outermembrane proteins [23–26]. In eukaryotic systems also, incorporation of a polar analog of leucine in vitro into the leucine-rich signal sequences of preprolactin and human placental prelactogen [26] has been shown to prevent translocation of these proteins. The need for an energized membrane state and appropriate membrane fluidity for the proper export and processing of proteins has been demonstrated [27–30]. Here, it has been shown that hydrophobic fragments of the signal sequence of chicken lysozyme bind to liposomes and are presumably associated with the hydrophobic core of the bilayer. Hence, interaction of signal sequences with the lipid component of membranes is likely to be important in the transfer of proteins across membranes. The membrane-modifying properties of signal sequences are being currently investigated.

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